October 10, 1956

Dear Dr. Dienes:

Thank you for your letter on Proteus 52. My brief examination of it was rather puzzling. Having learned a great deal from studying your Proteus strains, we are now entirely preoccupied with E. coli.

Some K-12 strains (including Y-10) give primary L-colonies quite readily when plated into the following medium: Agar, 1.0-1.2%; sucrose, 10%; $MgSO_4$ 0.2%; penassay broth, Difco; meat extract Difco 0.5%. Unlike Proteus, the coli L-form requires a rather high agar concentration, a fact which eluded and embarrassed us for some time. About 10% of the incubated bacteria form L-colonies, which seem to be of the B type.

We have not been very lucky so far in getting serial passages, although we have carried some four or five transfers. (We usually cut out a block of agar and mince it in some added broth (as above) with a Virtis tissue grinder.) There is negligible L-colony development on the surface of the agar. The progressive decrease in viability on passage suggests that there is a special growth factor, presumably synthesized by normal bacteria: we are looking along these and other lines to improve the medium. Serum is no help.

I am sending you Y-10 and an Hfr strain W-1895, which are an extremely fertile combination. The L-cycle plays no part in the usual conjugation process. I know that protoplasts of W-1895 are quite fertile. I have not been able to obtain, so far, a satisfactory L-colony development from W-1895, but there are a number of other Hfr strains which may be more satisfactory and are being tested. The experiment we are trying at the moment to to look for recombination between <u>two F-</u> strains, which cultures do not undergo conjugation in the bacterial form, as an index of new processes of genetic interaction. We have had one clean experimental trial so far, and the results were quite negative. We have to do more with varying conditions.

My own observations and conclusions agree with yours almost wherever they coincide. I hope the final paragraph of the (formerly missing) enclosu is a clearer statement of the protoplast-L colony relationship. However, I have not (with <u>E. coli</u>) seen the <u>segmentation</u> of a large body to form bacteria; instead, there is an elongation, and a bacterium is cut off, then proliferates. I have no fixed opinion about the mode of proliferation of the large body itself: your observations will certainly be an indispensable guide to my own. However, I am seeking to establish conditions where the entire cycle (in familiar material, E. coli) can be observed on the same specimens in living condition.

I have also noticed the tendency of nearly divided cells to swell from the point of division. Rhizobium's [illegible] of the role of fusion in the L-cycle must be based on the appearance of two-legged balloons (Mellon's

"zygospores"). But there are other better authenticated instances of fusion
de. .itely of*protoplasts or large bodies, though not associated with direct observations
eparated on further development. The question is whether this can have any genetic consequences, and whether it can occur between bacteria that would be unable to conjugate anyhow.

As I hope I have emphasized sufficiently, it is obvious that many pieces to this puzzle have been lying around already, and probably there are many more not yet recognized. The proposition (or definition if you like) that L-forms are protoplasts will at least have the virtue of attracting the attention of another group of investigators into this area.

You may be interested that Dr. B. Davis has found that bacterial mutants deprived of diaminopimelic acid will form near-protoplasts when deprived of DAP in a protective medium, otherwise lyse. DAP is of special interest as being uniquely found in bacterial cell walls. I have also communicated with Dr. Park and find he is in full agreement as to the significance of the uridine-diphosphate derivatives.

Yours sincerely,

Joshua Lederberg